Journal of Chromatography B, 976–977 (2015) 78–83

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

# Simultaneous determination of remimazolam and its carboxylic acid metabolite in human plasma using ultra-performance liquid chromatography–tandem mass spectrometry

Ying Zhou, Hongyun Wang, Ji Jiang, Pei Hu*

*Clinical Pharmacology Research Center, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China*

ARTICLE INFO

*Article history:*
Received 14 August 2014
Accepted 22 November 2014
Available online 2 December 2014

*Keywords:*
Determination
Remimazolam
Metabolite
Ultra-performance liquid chromatography–tandem mass spectrometry
Human plasma

ABSTRACT

A robust and validated method based on ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) has been developed for the simultaneous determination of remimazolam, which is a new chemical entity, and its major carboxylic acid metabolite (M1) in human plasma. Plasma samples were pre-purified by protein precipitation procedure and analyzed using an isocratic chromatographic separation over an Acquity UPLC CSH $C_{18}$ column. The mobile phase consisted of acetonitrile–water containing 10 mM ammonium formate and 0.1% formic acid at a flow rate of 0.4 mL $min^{-1}$. Positive electrospray ionization was employed as the ionization source in the multiple reaction monitoring (MRM) mode. The analysis time was about 1.5 min. The method was fully validated over the concentration range of 0.5–1000 ng $mL^{-1}$ for both analytes. The lower limit of quantification (LLOQ) was 0.5 ng $mL^{-1}$. Inter- and intra-batch precision was less than 8.4% and the accuracy was within 88.8–107.0%. The mean extraction recoveries obtained from three concentrations of QC plasma samples were 96.8%, 98.7% and 98.6% for remimazolam, 98.7%, 99.8% and 101.5% for M1, respectively. Selectivity, matrix effect and stability were also validated. The method was applied to the pharmacokinetic study of remimazolam in Chinese healthy subjects.



## 1. Introduction

Remimazolam is a short-acting GABA (A) receptor agonist that can be used as an intravenous sedative agent for potential use in day-case procedures, and the induction and maintenance of anesthesia [1,2]. As a member of the benzodiazepine class of drugs, remimazolam is designed to undergo rapid hydrolysis in the body by nonspecific tissue esterases to its pharmacologically inactive carboxylic acid metabolite (M1). Preclinical studies in sheep demonstrated that remimazolam produced a more rapid onset of action, and a shorter duration of action, compared with midazolam, which compatible with its potential human use as a short-acting i.v. sedative [3]. Also, a phase IIa clinical trial evaluating the procedural sedative effect for upper GI endoscopy in patients shows that the time to recovery from sedation was shorter and more consistent with remimazolam, compared to midazolam [2]. Therefore, because of its organ-independent metabolism and fast-acting onset and recovery, remimazolam appears to have potential advantages compared to other currently available short-acting sedatives.

Remimazolam Tosilate (Fig. 1(a)) was approved by China Food and Drug Administration as an investigational new drug for the potential use in day-case procedures, and the induction and maintenance of anesthesia in 2013 and is currently being evaluated in phase I trials. Remimazolam Tosilate is designed to undergo rapid hydrolysis in the body by nonspecific tissue esterases to its major metabolite M1. As we known, it is necessary to know about the exposure of the main metabolites considering their safety in human based on the FDA guidelines for industry safety testing of drug metabolites [4]. However, currently, few references are available about the determination of remimazolam or its main metabolites, except for one paper [5], that had mentioned an HPLC–MS/MS method to determine the concentration of remimazolam and its main metabolites in human plasma. The method described in this paper had less sensitivity, needed longer total run time, and required much larger plasma volume for sample preparation. Herein, we developed and fully validated a simple, sensitive and rapid method for simultaneous determination of remimazolam and its main metabolite (M1) in human plasma using only 50 μL

* Corresponding author. Tel.: +86 10 6915 6576; fax: +86 10 6915 8365.
*E-mail address:* zyyiva@163.com (P. Hu).

http://dx.doi.org/10.1016/j.jchromb.2014.11.022
1570-0232/

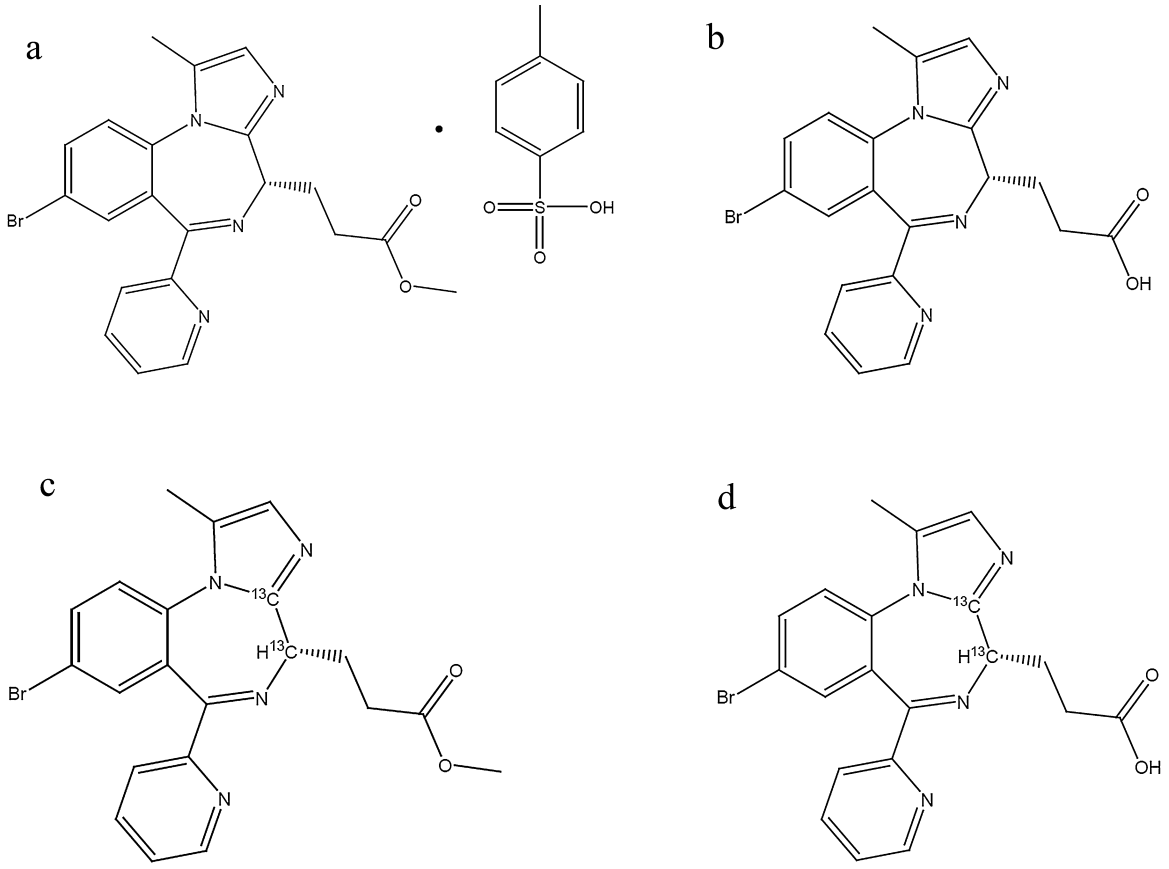


**Fig. 1.** Chemical structures of remimazolam Tosilate (a), M1 (b), [$^{13}C_2$] remimazolam (c), and [$^{13}C_2$] M1 (d).

plasma for each sample preparation. The run time was 1.5 min for each sample analysis, and the use of a stable isotope of the analyte as the internal standard (IS) was thought to be able to yield better assay performance results [6].

The objective of this study was to establish and validate an UPLC–MS/MS method with high sensitivity, accuracy and specificity for the simultaneous determination of remimazolam and its main metabolite in human plasma and to support the pharmacokinetic studies of remimazolam in Chinese healthy subjects.

## 2. Experimental

### 2.1. Chemicals and solvents

Remimazolam Tosilate and its internal standard [$^{13}C_2$] Remimazolam (Fig. 1(c)), carboxylic acid metabolite of remimazolam (M1, Fig. 1(b)) and its internal standard [$^{13}C_2$] M1 (Fig. 1(d)) were provided by Jiangsu Hengrui Medicine Co., Ltd. (Jiangsu, China). Methanol and acetonitrile was of chromatographic grade and obtained from Burdick & Jackson Lab. Ammonium formate and formic acid were of analytical grade and purchased from Sigma–Aldrich Co. LLC and Sinopharm Chemical Reagent Co., Ltd., respectively. Drug-free human plasma (anticoagulant: Heparin Sodium) was obtained from six different healthy subjects who were drug-free for at least two weeks. Distilled water was prepared with a Milli-Q water purifying system.

### 2.2. Calibration standard (CS) and quality control (QC) samples in human plasma

Stock solutions of remimazolam and M1 for CS and QC were prepared separately in methanol to a final concentration of 1 mg mL$^{-1}$ for each analyte and a mixed stock solution of IS (10 μg mL$^{-1}$) was also prepared in methanol. These stock solutions were further diluted to yield working solutions at several concentration levels. All the solutions were stored at −30 °C and brought to room temperature (25 °C) before use.

Calibration standards and QC samples in plasma were prepared by diluting corresponding working solutions with drug-free human plasma, respectively. The final concentrations of calibration standards were 0.5, 2, 5, 20, 50, 200, 500 and 1000 ng mL$^{-1}$. The final concentrations of QC samples for the evaluation of intra- and inter-batch precision and accuracy, recovery and matrix effect and stability were 1.5, 30 and 750 ng mL$^{-1}$. Mixed IS working solution (100 ng mL$^{-1}$) was prepared with methanol. Plasma samples were stored at −70 °C.

### 2.3. Sample preparation

All the plasma samples were pretreated by protein precipitation procedure. Plasma sample (50 μL) was spiked with 150 μL of IS solution (100 ng mL$^{-1}$). The mixture was vortex-mixed for 3 min and centrifuged at 16,242 × *g* for 5 min, and then 150 μL of the supernatant were collected and evaporated to dryness under a stream of nitrogen at 40 °C. The residues were reconstituted in 100 μL of acetonitrile/water containing 10 mM ammonium formate and 0.1% formic acid (40:60, v/v) and vortex-mixed for 1 min and then centrifuged at 16,242 × *g* for 1 min before injection. Finally, 7.5 μL of the dissolved sample was injected to the UPLC–MS/MS system.

### 2.4. Liquid chromatography–tandem mass spectrometry

Plasma samples were analyzed using Acquity UPLC Core system coupled with Xevo-TQS triple quadrupole mass spectrometer